Sambhar Salt Lake

Chemical composition of the brines and studies on haloalkaliphilic archaebacteria

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Received September 26, 1989/Accepted August 8, 1990

Abstract. The saline and alkaline brines from the Sambhar Salt Lake (SSL), both from the main lake and from the solar evaporation pans at Sambhar Salt Limited, Sambhar, Rajasthan, India, were studied with respect to their chemical composition and presence of red, extremely haloalkaliphilic archaebacteria. The brines had pH values of 9.5 ± 0.2 and a total salt content ranging from 7% (w/v) to more than 30% (w/v). Sodium chloride, sodium carbonate, sodium bicarbonate and sodium sulphate were the principal salts present in these brines which lacked divalent cations (calcium and magnesium). Six strains of red, extremely haloalkaliphilic bacteria, designated SSL 1 to SSL 6, were isolated. All the isolates showed obligate requirements for sodium chloride (> 15%, w/v) and high pH (> 9.0). Magnesium ions were required in traces for maintaining morphological structure and pigmentation. All these strains possessed the diether core lipids, phosphatidylglycerol (PG), phosphatidylglycerophosphate (PGP), and bacterioruberins characteristic of halophilic archaebacteria. The strains were assigned to the newly proposed genus Natronobacterium.

Key words: Alkaline saline environment — Sambhar Salt Lake — Haloalkaliphilic bacteria — Archaea bacteria — Natronobacterium — Natronobacterium SSL 1 (ATCC 43988) — Natronobacterium SSL 6 (ATCC 43987) — Dunaliella salina

Chemical analyses of the brines collected both from the main lake as well as the solar evaporation pans (locally “kyars”) of the saline and alkaline Sambhar Salt Lake (SSL), Sambhar, Rajasthan, India. The lake is situated at 26° 58’ N, and 75° 5’ E, in the middle of a closed depression in the Aravalli schists, approximately 65 km northwest of Jaipur, with its axis northwest to southeast (Aggarwal 1951). Six strains of red, extremely haloalkaliphilic bacteria were isolated from the high density brines, and characterized.

Materials and methods

Organisms

Natronobacterium gregoryi (NCMB 2189) and N. magadii (NCMB 2190) were provided as gift by Dr. H. N. M. Ross and Dr. W. D. Grant, UK. Strains SSL 1 to SSL 6 were isolated from the SSL brines.

Chemical analyses

Chemical analyses of the brines collected in July, 1984 were carried out at the laboratory of Gujarat Water and Air Pollution Board, Ahmedabad, India, by methods of Taras et al. (1980). Sodium and potassium were determined by the flame photometric method at wavelength 589 nm and 776.5 nm, respectively. Calcium and hardness were determined by EDTA titrimetric method. Magnesium was estimated by calculating the difference between hardness and CaCO₃ content. Chloride was determined by an argentometric method. Sulphate was determined turbidometrically as BaSO₄. Car-
bonate was determined by titrimetric estimation of hardness and the alkalinity was reported as CaCO₃. Bicarbonate was determined by titration against 0.02 N H₂SO₄ using phenolphthalein and methyl orange as indicators. Total dissolved solids were determined by a gravimetric method, using 50 ml of filtrate obtained by filtering the brine sample using Whatman filter paper No. 42. Suspended solids were determined by calculating the difference between total solids and total dissolved solids. The density (d) of the brine was measured using a heavy liquid densitometer at room temperature (35° ± 1°C). pH was measured using a Systronics pH meter 324.

**Enrichment and isolation**

Enrichment cultures were obtained in modified Brown medium as described by Tindall et al. (1980). A brine sample (2%, w/v) was inoculated into 100 ml of the broth dispensed in 300-ml Erlenmeyer flasks and incubated on a shaker at 37°C for 7–10 days (Mullakhanbhai and Larsen 1975) Pure cultures were obtained on agar plates prepared by the addition of 2.5% (w/v) Difco agar to the medium. The isolates were maintained on slants at 5 ~ 10~ agar plates prepared by the addition of 2.5% (w/v) Difco agar to the medium. The isolates were maintained on slants at 5°–10°C, pH 9.5.

**Growth experiments**

The strains were grown in media, pH 9.5, in which the concentrations of NaCl, MgSO₄, 7H₂O, and KCl varied. Varying amount of KCl was determined by titrating against 0.25 mm-thick, preparative 0.75-mm-thick) in the solvent system: chloroform-methanol-acetic acid-water (85:22.5:10:4; by volume). Lipids were detected by the following spray reagents: (NH₄)₂MoO₄·HClO₄ for phospholipids; 0.5% x-naphthol-H₂SO₄ for glycolipids; H₂SO₄-ethanol (1:1; by volume) followed by charring for detection of all lipids (Kates 1972; Kushwaha et al. 1982).

**Light microscopy**

A Carl-Zeiss light microscope was used with phase-contrast and dark-field optics.

**Biochemical tests**

Routine biochemical tests were performed using modified Brown medium as described by Holding and Collee (1971), Gonzalez and Gutierrez (1970) and Tindall et al. (1984). Chemicals used were of analytical grade.

The utilization of carbon sources including glucose, fructose, lactose, maltose, mannitol, ribose, sucrose and xylose was tested in v/v) by the method described by Gochnauer et al. (1972). Absorption spectra were taken in a Beckman DU-40 UV-Vis double beam spectrophotometer.

**Lipid analyses**

Whole-organism methanolysate was prepared and the diether core lipids were extracted in hexane as described by Minnikin et al. (1975). Diether core lipids were detected using a thin-layer chromatographic method (Ross et al. 1985). Lipid analyses were performed using modified Brown medium as described by Tindall et al. (1980). A brine sample (2%, w/v) was inoculated into 100 ml of the broth dispensed in 300-ml Erlenmeyer flasks and incubated on a shaker at 37°C for 7–10 days (Mullakhanbhai and Larsen 1975) Pure cultures were obtained on agar plates prepared by the addition of 2.5% (w/v) Difco agar to the medium. The isolates were maintained on slants at 5°–10°C, pH 9.5.

**G + C mol% determination**

The halobacterial DNA was prepared by the modification of the method of Marmur (1961). The lysozyme treatment step was omitted, as the cells lysed in hypotonic solutions. The G + C content of the DNA preparations in 0.012 M and 0.12 M phosphate buffer was determined from its melting point by the method of Marmur and Doty (1962), at Biochemical Division, National Chemical Laboratory, Pune, India. The wavelength scanning of the DNA solution was carried out in the range 220 to 320 nm on a Shimadzu double beam spectrophotometer model UV-210 A.

**Results**

**Chemical composition**

The brines collected from the Sambhar Salt Lake in July, 1984 had a total salinity of ~ 7% (w/v) (~ 1.035, d) and the concentration of salts in the brines varied from 12 to 30% w/v. The lake dries up during the hot summer season (45°–45°C, atmospheric temperature) and the salt crystallizes on the surface of the lake bed. The principal ions contributing to the salinity of the brines, which have pH 9.5–10.5, were sulphates, carbonates, bicarbonates, chlorides, sodium and smaller amounts of potassium (Table 1). These ions accounted for up to 99% of the total salts. However, the brines lacked the divalent cations, calcium and magnesium. The brines were rich in suspended solids (0.7–2.8% w/v).

**Ecological and microbiological observations**

The brines of Sambhar Salt Lake appeared light green after the monsoons. The lake brine (1.035–1.042, d) flows into the various condensers and crystallizer pans (kyars) located at Jhapog, Gudha, Nawa (New), Main Line and Deodani. The colour of the brines varied from yellow to green and red to pink, depending on their densities. The low density (1.074–1.115, d) brines at